Cortical and subcortical brain morphometry differences between patients with autism spectrum disorders (ASD) and healthy individuals across the lifespan: results from the ENIGMA-ASD working group

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ABSTRACT

Objective:
Neuroimaging studies show structural differences in both cortical and subcortical brain regions in children and adults with autism spectrum disorders (ASD), compared to controls. Findings are inconsistent, however, and it is unclear how differences develop across the lifespan. We aimed to investigate brain morphometry differences between participants with ASD and controls cross-sectionally across the lifespan, in a large worldwide sample from the ENIGMA-ASD Working Group.

Methods:
The sample comprised 1571 patients with ASD and 1651 healthy controls from 49 participating sites (age range: 2-64 years). MRI scans were preprocessed at individual sites with a harmonized protocol based on validated, automated segmentation software. Mega-analyses were used to test for case-control differences in subcortical volumes, cortical thickness and surface area. Development of brain morphometry over the lifespan was modeled using a fractional polynomial approach.

Results:
The case-control mega-analysis demonstrated that ASD was associated with smaller subcortical volumes of the pallidum, putamen, amygdala and nucleus accumbens (effect sizes d=0.11 to d=0.16), as well as increased cortical thickness in the frontal cortex and decreased thickness in the temporal cortex (effect sizes d=0.20 to d=-0.21). Analyses of age effects indicate that the development of cortical thickness is altered in ASD, with the largest differences occurring around adolescence. No age by ASD interactions were observed in the subcortical partitions.

Conclusions:
The ENIGMA-ASD working group provides the largest study of brain morphometry differences in ASD to date, using a well-established and validated publicly available analysis pipeline. ASD subjects showed altered morphometry in the cognitive and affective parts of the striatum, frontal cortex and temporal cortex. Complex developmental trajectories were observed for the different regions, with a developmental peak around adolescence. Our findings suggest an interplay in the abnormal development of the striatal, frontal and temporal regions in ASD across the lifespan.

KEYWORDS: ASD, brain volumes, cortical thickness, imaging, life span, mega-analysis

Introduction

Autism Spectrum Disorder (ASD) is a relatively common childhood onset neurodevelopmental disorder, affecting about 1.4% of the population (1–3). ASD is usually diagnosed before age 6, and often leads to lifelong problems in social adaptation and impaired functioning. Although ASD is highly heritable and considered to be a brain-based disorder, the biological underpinnings of the disorder and its development over the lifespan remain largely unclear.

Much research has focused on the role of anatomical brain abnormalities in ASD (4–7). Both larger (7) and smaller (8) volumes of striatal structures have been reported in ASD, as well as smaller hippocampal (9) and – in childhood – larger amygdala volumes (10). Increased intracranial volume (11), total grey matter and cortical thickness have also been reported in ASD (12), with more specific cortical effects observed mainly in the frontal (13) and temporal lobes (14). These structural abnormalities play a crucial role in current theories on the neurobiology of autism. Specifically, altered frontal and striatal volumes and disrupted fronto-striatal connectivity are key components in the executive function deficit theory of ASD (15–17). On the other hand, abnormal amygdala volume, specifically in childhood (18; 19), plays a central role in the social theories of ASD (10; 20; 21).

However, existing neuroimaging studies report considerable heterogeneity in the direction and effect size of these morphometric brain differences (12; 22; 23), with a recent large scale study by Haar et al. even indicating overall increased grey/white matter measures, but very small local effects of ASD on brain morphometry (12). This heterogeneity in the literature may be due to various factors. Firstly, variation in case-control differences of brain structures may be due to age differences between study samples. Prior research suggests altered patterns of cortical and subcortical development in ASD, generally reporting abnormally higher volumes in childhood followed by a more rapid volumetric decline during adolescence and adulthood (13; 24–26). Secondly, factors like sex, medication use, symptom severity and presence of comorbidities may also affect case-control differences in brain structures. Furthermore, methods based factors such as variation in data acquisition, processing and analysis protocols may influence the results reported across different studies.

Several recent studies have used the anatomical differences in ASD as the basis for multivariate analyses with the ultimate goal of using these differences as a tool for categorizing ASD (12; 27; 28). These efforts, however, remain yet to be validated in clinical settings (29). The heterogeneity of anatomical differences in the various samples may also underlie the lack of consistent results in this line of research.

The current study addresses several of these issues. Specifically, the current collaboration was established as part of ENIGMA, the world-wide imaging genetics consortium aimed at unifying analyses methods across a range of neuropsychiatric disorders. We use the ENIGMA processing and analysis pipelines to merge individual subject data from 49 existing ASD case-control cohorts (of which 16 cohorts were collected previously as part of the ABIDE consortium (6), and 17 as part of ABIDE –II (30)) to determine whether and which changes in subcortical volumes as well as cortical thickness and surface area underlie the ASD phenotype across the lifespan. By unifying processing and analysis, we were able to eliminate a large part of the methodological noise between individual studies. Additionally, we were able to investigate directly the effects of sex, IQ and symptom severity across this extensive sample. Last but not least, studies employing small sample sizes are liable to overestimate effect sizes (31). With 43 of the 49 currently included cohorts employing sample sizes of less than 100 subjects, it is important to test whether results of small scale studies remain robust within this large cohort.

Based on existing literature, we expect subjects with ASD to show smaller subcortical volumes specifically in the putamen, caudate, but larger volumes in hippocampus and amygdala. We furthermore expect generally increased grey matter volumes and cortical thickness in subjects with ASD, specifically we expect increased thickness in frontal and temporal cortices (32). Given the broad age range of the current sample, we also charted in detail the development of these morphological features over the lifespan in ASD, albeit based on cross-sectional data. Based on previous studies, we expect the largest effects of ASD during childhood with normalization of features over adolescence and adulthood (13; 24–26)

Methods

Contributing sites

The ENIGMA-ASD working group is an open cohort, aimed at bringing together MRI data from a wide range of ASD studies. The working group was started in 2015, and remains open for any new groups working with ASD patients of any age. The working group implemented a data freeze to execute the current subcortical volume analyses in March 2017, at which point we included a total of 1571 patients with ASD and 1651 healthy controls from 49 participating research groups, spanning 13 countries. Both the ABIDE and ABIDE-2 consortia were included in the current cohort (6; 30). Demographic information for all participants may be found in **Table 1** and **Figure 1**, details of the different contributing samples can be found in **Supplementary Table 1.** All contributing sites had local ethical approval for the sharing of meta-analytic test statistics, 48 out of 49 sites had approval for sharing anonymized individual data. Even though we included samples from the entire ASD spectrum, the vast majority of the included individuals did not have an intellectual disability, hence the comparable IQ scores between patients and controls.

(Insert **Table 1** here)

(Insert **Figure 1** here)

FreeSurfer segmentation

Structural T1-weighted MRI scans acquired at different contributing sites were segmented using standardized and publically available ENIGMA imaging protocols (http://enigma.ini.usc.edu/protocols/imaging-protocols/). These automated protocols based on Freesurfer5.3 segmentations are fully validated, to allow maximal uniformity and comparability across sites. For each participant, left and right subcortical volumes, cortical thickness and cortical surface area measures were calculated. The mean of left and right volumes were used for most subsequent analyses. Standardized quality control depended on the automatic detection of segmentation outliers for each volume, followed by visual inspection of outlying volumes. Poorly segmented regions were removed from further analyses. Detailed information on the QC procedure can be found in the supplementary information.

Case-control analyses

The main aim of this study was to investigate differences in (sub)cortical morphometry related to ASD status. To accomplish this, individual segmentation subcortical volumes, cortical thickness and cortical

surface area were merged for participants over all sites into one “mega-analysis”. The effect of diagnosis (patient/control) on each ROI was calculated using a linear mixed effects model (nlme package in R) including a polynomial fit for age, sex and IQ as fixed factors, and age*diagnosis and age*sex interactions. For subcortical volumes, total ICV was added as a fixed factor. Scan site was added as a random factor. P-values were corrected for multiple comparisons using the false discovery rate (FDR) adjustment.

Several additional sensitivity analyses were performed to investigate how sex, IQ, medication use, comorbidities, ASD severity, hemisphere effects or total ICV differences might have influenced the main effect of ASD status on (sub)cortical measures. Age and sex information were available for all incorporated studies. For IQ, medication use, comorbidities and ASD severity, data were available for some samples (see supplement for the available data per site). Given the large variation in available detail on medication use and comorbidity assessment, these were included in the analyses as a dichotomous measure (current medication vs no medication use, current comorbidity vs no comorbidities). For ASD severity, the most ubiquitously available measure was the total ADOS_G score (33), which was used as an estimate of ASD severity. For a more detailed comparison to the Haar et al. (12) study, a permutation based post-hoc analysis was performed.

To allow for comparisons across the different sites as well as to control for any unobserved effects that may influence the mega-analysis, we repeated the same analysis of the diagnosis effect in a more conservative meta-analysis, running a linear regression model for each site separately. The I^2 statistic (34) was calculated to estimate the heterogeneity of the diagnostic effects across sites, indicating the percentage of variation across studies that is due to heterogeneity rather than chance.

Age effects modeling

Given the importance of developmental trajectories when estimating the effects of ASD, we used a fractional polynomial approach to estimate the optimal fit for development of the volume, thickness or surface area with age (mfp package in R) (35). For all ROIs that showed a significant effect of age or age*diagnosis interaction in the mega-analysis, the optimal model was estimated for ASD subjects and healthy controls using one- and two-term curvilinear models, choosing the best fitting model out of 44 possible two-term models - with possible powers of -2, -1, -0.5, 0, 0.5, 1, 2 and 3 (these models also included sex, IQ, and scan-site as covariates).

Power Calculation

Using G*Power version 3.2.1, we calculated the minimal effect sizes observable given 1,571 participants with ASD and 1,651 healthy controls. At a minimum desired power level of 0.8 and p-value of 0.05 (two-tailed), we have power to observe effects of d>0. 108 (Cohen’s d).

Results

Case-control differences in subcortical and cortical partitions

Both sex and IQ were not distributed equally between the subjects with ASD and healthy controls. There were proportionally more females in the control than ASD group (23.8%, as compared to 14.25%; p<.001) and controls had a significantly higher mean IQ (111 vs 103, p<.001). Both sex and IQ were incorporated as covariates in the main mega-analysis to correct for these differences. Additional sensitivity analyses without correction for IQ as well as analyses with subgroups balanced on sex and IQ can be found in the supplementary information. The effects of medication use and associations

with symptom severity were also investigated.
The results of the subcortical mega-analysis are presented in **Table 2** and **Figure 1.** Even though the hippocampus is technically a cortical area we have chosen to add hippocampal volume to the subcortical results, since it is segmented as a subcortical volume in Freesurfer. ASD was associated with significantly smaller mean volumes of the putamen (p<.001, d=-0.10), pallidum (p<.05, d=-0.08) amygdala (p<.05; d=-0.08) and nucleus accumbens (p<.001, d=-0.13), as well as larger mean volumes of the lateral ventricles (p<.001, d=0.11) and ICV (p<.001, d=0.13). Additional sensitivity analyses correcting the subcortical volumes for intracranial gray matter volume instead of total ICV are added to the supplementary materials.
The effects of ASD diagnosis on cortical thickness are presented in **Table 3**. We observe significantly increased overall cortical thickness (p<.003, d=0.41) in subjects with ASD, as well as more specifically in 9 of the 34 cortical partitions, located in the middle and superior frontal, orbitofrontal, inferior frontal and posterior cingulate areas. Inversely, decreased cortical thickness was observed in subjects with ASD in 7 partitions, located in the temporal, entorhinal and parahippocampal areas (see **Figure 2**). We also found increased overall grey matter volume (p<.052, d=0.23) among subjects with ASD. No effects of ASD diagnosis on cortical surface area were found.

(Insert **Figure 2** here)

(Insert **Table 2** here)

Post hoc analyses per hemisphere indicated that for the lateral ventricles (left p<.004; right p<.006), putamen (p<.03, p<.05) and nucleus accumbens (p<.008; p<.02) both hemispheres contribute to the overall effect. For the hippocampus and amygdala only the right hemisphere showed a significant effect (p<.05; p<.03). Increased cortical thickness in subjects with ASD was observed in frontal cortex for both hemispheres, as well as decreased thickness in the temporal cortex. In the right hemisphere, significantly thicker cortex in ASD was furthermore observed in the cingulate cortex, and decreased thickness in the parietal cortex (**see Supplementary Table 8**).

To investigate differential effects between sites, a meta-analysis was performed by treating every site independently and aggregating the results. The results from the case-control meta-analysis confirmed smaller pallidum (p<.04, d=-0.09), amygdala (p<.03, d=-0.09) and nucleus accumbens (p<.01, d=-0.1) volumes in ASD; as well as higher volumes of the lateral ventricles (p<.003, d=0.13) and ICV (p<.016, d=0.06). The meta-analysis also showed increased cortical thickness in subjects with ASD in 3 out of 11 of the frontal partitions, and decreased thickness in 8 out of 9 temporal partitions, as well as in the supramarginal gyrus (**see Supplementary Table 2).** Overall, the meta-analysis showed smaller effect sizes and higher standard error of effect sizes than the mega-analysis. The I^2 test indicates moderate to high heterogeneity across sites for all effect sizes (I^2=15.19–64.63). Individual test statistics for the case-control comparison per site are listed in the supplementary materials (**see Supplementary Table 12**).

Age effects
Main linear effects of age were observed for all subcortical volumes (**see Table 2**). However, no interaction effects between diagnosis and age were found. We calculated fractional polynomial fits for the age effect for all the above mentioned ROIs, estimating the polynomial fit for these volumes

(see **Figure 3** and **Supplementary Table 11**). The fractional polynomial approach indicates that the optimal model for the age effect in all subcortical volumes contains the powers of 0.5 and 2 (see **Figure 2**). These results indicate that the developmental curves of the subjects with ASD and healthy controls follow similar trajectories over time, which confirms the observed lack of detectable age by diagnosis interactions.

Main linear effects of age on cortical thickness were observed in 30 out of 34 partitions, all of them showing a negative relation between age and thickness (**see Table 1**). The 4 partitions not showing a significant effect were all overlapping with the partitions showing a negative relation between thickness and ASD diagnosis (temporal, entorhinal and parahippocampal areas). A quadratic effect of age was observed in the insula. Age by diagnosis interactions were further observed in 24 of the partitions that also showed a linear age effect. Fractional polynomial plots were calculated for these partitions as well, showing complex developmental curves including both quadratic and cubic effects across the partitions (**see Figure 3**). These visualizations indicated that subjects with ASD show a peak in cortical thickness differences around adolescence, with both the greater thickness in the frontal cortex and lesser thickness in the temporal cortex peaking around this age.

(insert **Figure 3** here)

Sex and IQ effects

The mega-analysis shows significant effects of sex on ICV, lateral ventricles, thalamus, caudate, putamen, amygdala and nucleus accumbens volumes, indicating larger volumes in males than females. No interactions between diagnosis and sex were observed. Effects of IQ on brain volumes were observed for the putamen, hippocampus, amygdala and nucleus accumbens volumes, with larger volumes associated with a higher IQ (see **Table 2**). The three-way interaction of diagnosis, age and sex rendered no significant results.

Effects of sex on cortical thickness were observed in transverse temporal, caudal-middle frontal and superior frontal partitions, all of them indicating thicker cortex in males. Subjects with a higher IQ showed greater cortical thickness in precentral and rostral anterior cingulate partitions, and lower thickness in medial orbitofrontal and caudal anterior cingulate partitions.

Medication, comorbidity and symptom severity effects

Within subjects with ASD, further mega-analyses were performed to test for any effects of medication use, comorbidities and ASD symptom severity on subcortical volumes (see supplementary materials). Neither medication use nor the presence of comorbidities significantly influenced subcortical volumes within this sample. Cortical thickness was associated with medication use only in the inferior-temporal partition ($d=-0.47$, $p<.002$), but not with comorbidity. ASD symptom severity analyses showed that higher ADOS scores were associated with larger ICV and lateral ventricles, and lower volumes in putamen, nucleus accumbens, thalamus, amygdala and hippocampus (see **Supplementary Table 9**). Greater thickness in the frontal areas and lower thickness in the temporal areas was associated with higher ADOS scores. Interestingly, higher ADOS scores were additionally associated with increased thickness in cingulate, parietal and occipital regions, while lower cortical thickness was associated with higher ADOS scores in the insula.

Discussion

We investigated subcortical brain volumes, cortical thickness and surface area in the largest sample to date of participants with ASD and typically developing controls in a wide age range. ASD was found to be associated with significantly smaller volumes of putamen, pallidum and nucleus accumbens, but larger volumes of the lateral ventricles. Subjects with ASD also showed generally larger ICV, total GM and total cortical thickness, but no differences in surface area. Our analyses indicate a split in the direction of cortical thickness effects between the frontal and temporal cortices, where subjects with ASD showed increased cortical thickness in the frontal cortex, but decreased thickness in the temporal cortex. The effect sizes of these cortical and subcortical group differences ranged from d=-0.21 to 0.20, indicating small to moderate effects and significant overlap in the distribution of brain morphometry measures between participants with ASD and healthy controls, in line with effect sizes found in the ENIGMA-ADHD, Schizophrenia and Bipolar disorder working group findings (36–38).

Increased cortical thickness in general, as well as increased lateral ventricle volumes, are all in line with previous results found in the meta-analysis by Haar et al. (12), although they do not find any alterations in the basal ganglia volumes or frontal cortical thickness. Decreased temporal cortical thickness and increased frontal thickness however were observed in another recent large scale meta-analysis on brain morphometry in ASD (39). Our sensitivity analyses based on the permutation testing used in the Haar et al. (12) study indicate that the discrepancies with the current results can mainly be attributed to differences in sample size, as we replicate prior results using the permutation method on our larger cohort sample. This comparison further indicates the necessity of large scale cohort studies, and stresses the caution needed when interpreting effects with these limited effect sizes.

The involvement of pallidum, putamen and nucleus accumbens indicates an important role for the socio-motivational and cognitive and motor systems of the striatum in the neurobiology of ASD (40). Taken together with the increased cortical thickness in the frontal cortical regions, which are mainly involved in cognitive control, these findings are in line with prior studies relating aberrant frontal-striatal connectivity to the repetitive behavior and executive functioning deficits observed in ASD (6; 14; 17; 41). Nucleus accumbens deficits have additionally been suggested to support the theory of social reward based differences underlying part of the behavioral phenotype in ASD (15). In contrast to some earlier findings (19), but in line with others (10), we also find a slightly smaller amygdala volume in ASD. Further research is necessary to investigate whether nucleus accumbens and amygdala volume changes are related to social and reward processes in ASD. Decreased thickness in the temporal cortex in ASD may be further related to both social (42) and language deficits in ASD (43). Our post-hoc analyses on ASD severity further indicate that cortical and subcortical morphometry is related to ASD severity, following the same direction as the group effect, indicating that these alterations indeed serve a functional role in the ASD etiology.

The age distribution in the current sample ranged from 2 to 64 years, providing an unprecedented cross-sectional view of the development of brain morphometry in ASD and healthy controls over the lifespan. Our subcortical results indicate complex - linear and quadratic - age effects in thalamus, hippocampus, amygdala, nucleus accumbens and lateral ventricles. The fractional polynomial fits for these volumes indicate that over all groups the subcortical volumes follow a quadratic growth model with a distinct peak around puberty, which is in line with previous literature on development of these

regions (44). As opposed to some prior studies and reviews (e.g. (26)), no evidence was found for age*diagnosis interaction effects in any of the investigated subcortical volumes (45). Cortical thickness, on the other hand, showed large scale effects of both age and age*diagnosis interaction. We observed a general declining cortical thickness over age, in line with previous literature (13; 32). However, as compared to controls, subjects with ASD in general showed strongest group differences during childhood and adolescence, with normalized or even reversed thickness results in adulthood. Interestingly, this was observed both for the increased thickness in ASD in the frontal areas as well as the decreased thickness in the temporal lobe. These results indicate a complex maturation pattern for the subcortical, frontal and temporal structures in ASD, peaking around adolescence. Specifically, the balance of frontal, temporal and striatal maturation may prove a valuable marker for the development of ASD symptoms and treatment response, though further longitudinal studies are required to verify the predictive validity of these morphometric measures.

Our findings also replicate previously reported main effects of sex (46) and IQ (47) on brain volumes, with generally larger subcortical volumes and increased thickness found in males and in participants with a higher IQ. The mega-analytic approach can statistically correct for differences in sex and IQ between participating sites, removing some of the outcome variance associated with different distributions of these factors between sites. We observed that males have, on average, larger basal ganglia volumes and increased cortical thickness, but patient groups had a larger proportion of males, indicating that sex could not have confounded the ASD effect. Although we find no evidence of sex by diagnosis interaction, the increased volumes and thickness in both males and females with ASD could be taken as evidence for the 'extreme male brain' hypothesis, (48), though this study was not optimally designed to validate this hypothesis, while other recent large scale studies have found considerable evidence for gender effects in brain morphometry in ASD (49). We aim to replicate the analysis by Ecker et. al. in a future publication of the ENIGMA-ASD cohort. Neither medication use nor comorbidity had any large scale influence on brain morphometry in ASD. Within the ASD sample, overall effects of symptom severity were largely consistent with the direction of the between-group effects. This supports the inference that the observed differences in morphometry are indeed related to the phenotypic expression of ASD in this cohort.

Since individual participant data was available for almost the entire sample, we could compare meta- and mega-analytic approaches in this dataset. The main difference between these approaches is that in a meta-analysis, within and between group variance is estimated for each separate site, while for the mega-analysis, variance is estimated within-group. Our results indicate that the meta-analysis is generally less sensitive to group differences, with smaller effect sizes and higher standard errors found in the meta-analysis. The meta-analysis also allowed us to investigate the effects of ASD on brain morphometry per site (as seen in the supplementary results). This analysis indicates significant heterogeneity in the direction and size of the effects between participating sites. The heterogeneity of effect sizes as expressed with the i^2 measure was moderate to high for all volume differences found. This heterogeneity is very important to take into account when interpreting single sample studies. Even though all the data in the current study were processed using the same analysis and QC pipeline, and were corrected for effects of age, sex and IQ identically across sites, we still find significant differences in the estimated main effects between sites. Some of these differences may be due to random sampling differences of the ASD population, in particular within the smaller samples. However, this is less likely to be the case in larger samples employing hundreds of subjects.

Alternatively, the within-group heterogeneity might be indicative of different biological mechanisms or subtypes underlying ASD, and may therefore be informative for further classification studies. In any case, the existence of this heterogeneity underlines the importance of large scale studies such as ENIGMA to develop reliable benchmarks for the different major psychiatric disorders. The establishment of these benchmarks allows us to more accurately tackle the heterogeneity due to measurement differences and biological differences in neurodevelopmental studies. Planned multivariate factor analyses and subtyping analyses may additionally provide further insight into different biological mechanisms in ASD.

One of the main goals of the ENIGMA consortium is to unify analysis methods not only over samples, but also over different disorders. The recent publication of the ENIGMA-ADHD working group is based on the same analysis pipeline and mega-analysis as the current study, using a similarly sized sample of participants with ADHD and controls (38). The ENIGMA-ASD and ADHD results suggest similar decrease of volumes in the putamen, amygdala and nucleus accumbens, whereas differences are found in the pallidum volume in ASD but not ADHD. Additionally, age analyses of subcortical volumes using fractional polynomials suggest different patterns of neural development in ASD and ADHD. Whereas subjects with ASD show similar volume growth curves as controls, subjects with ADHD showed a significant diagnosis by age interaction, with different developmental models most clearly seen in nucleus accumbens and putamen volumes for subjects with ADHD (38). These partly overlapping striatal volume differences offer a fascinating starting point for further investigation of the shared and unique neurobiological underpinnings of both ADHD and ASD, as direct comparisons between these two cohorts have not yet been completed at the time of writing.

Some limitations should be considered when interpreting these findings. Primarily, our different participating sites used different scanners and acquisition protocols. Though we have controlled for the effects of scan site in our models, we cannot fully exclude potential influence of these measurement protocols on the data. We were also unable to obtain longitudinal data for the current samples, which prohibits a within-subject analysis of brain development in ASD. Future large scale efforts should in our opinion be aimed at also standardizing acquisition protocols and long term follow ups.

To conclude, this study showed the abnormal development of cortical thickness and subcortical volumes in ASD in the largest sample to date, as obtained by the ENIGMA-ASD working group. We observed smaller volumes of putamen, amygdala, nucleus accumbens and pallidum, increased frontal cortical thickness and decreased temporal cortical thickness in ASD compared to controls. Our age analyses, show that subcortical differences in ASD remain relatively stable over the lifespan, whilst cortical alterations in ASD show a peak in childhood and early adolescence, and taper off over adulthood. Future functional activation and resting-state connectivity studies will want to take into account these differences in maturation, and focus on unraveling how the balance between frontal, temporal and subcortical alterations influences the expression of the ASD phenotype across the lifespan. No differences in the development of brain morphometry was observed between males and females with ASD.

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Declaration of interest. Jan K. Buitelaar has been in the past 3 years a consultant to / member of advisory board of / and/or speaker for Janssen Cilag BV, Eli Lilly, Lundbeck, Shire, Roche, Medice, Novartis, and Servier. He has received research support from Roche and Vifor. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties.
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All other authors have no interests to declare.

References

1. Baird G, Simonoff E, Pickles A, Chandler S, Loucas T, Meldrum D, Charman T: Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). Lancet 2006; 368:210–215
2. Christensen DL, Baio J, Braun KV, Bilder D, Charles J, Constantino JN, Daniels J, Durkin MS, Fitzgerald RT, Kurzius-Spencer M, Lee L-C, Pettygrove S, Robinson C, Schulz E, Wells C, Wingate M, Zahorodny W, Yeargin-Allsopp M: Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years--Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. Surveill. Summ. 2016; 65:1–23
3. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. 2013.
4. Amaral DG, Schumann CM, Nordahl CW: Neuroanatomy of autism. Trends Neurosci. 2008; 31:137–145
5. Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, Morgan J: Mapping early brain development in autism. Neuron 2007; 56:399–413
6. Di Martino A, Yan C-G, Li Q, Denio E, Castellanos FX, Alaerts K, Anderson JS, Assaf M, Bookheimer SY, Dapretto M, Deen B, Delmonte S, Dinstein I, Ertl-Wagner B, Fair D a, Gallagher L, Kennedy DP, Keown CL, Keysers C, Lainhart JE, Lord C, Luna B, Menon V, Minshew NJ, Monk CS, Mueller S, Müller R, Nebel MB, Nigg JT, O'Hearn K, Pelphrey K a, Peltier SJ, Rudie JD, Sunaert S, Thioux M, Tyszka JM, Uddin LQ, Verhoeven JS, Wenderoth N, Wiggins JL, Mostofsky SH, Milham MP: The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol. Psychiatry 2014; 19:659–67
7. Turner AH, Greenspan KS, van Erp TGM: Pallidum and lateral ventricle volume enlargement in autism spectrum disorder. Psychiatry Res. Neuroimaging 2016; 252:40–45
8. Sussman D, Leung RC, Vogan VM, Lee W, Trelle S, Lin S, Cassel DB, Chakravarty MM, Lerch JP, Anagnostou E, Taylor MJ: The autism puzzle: Diffuse but not pervasive neuroanatomical abnormalities in children with ASD. NeuroImage Clin. 2015; 8:170–179
9. Groen W, Teluij M, Buitelaar J, Tendolkar I: Amygdala and hippocampus enlargement during adolescence in autism. J. Am. Acad. Child Adolesc. Psychiatry 2010; 49:552–560
10. Bellani M, Calderoni S, Muratori F, Brambilla P: Brain anatomy of autism spectrum disorders II: Focus on amygdala. Epidemiol. Psychiatr. Sci. 2013; 22:309–312
11. Fombonne E, Rogé B, Fombonne E, Roge B, Claverie J, Courty S, Fremolle J: Microcephaly and Macrocephaly in Autism Microcephaly and Macrocephaly in Autism. 1999;
12. Haar S, Berman S, Behrmann M, Dinstein I: Anatomical Abnormalities in Autism? Cereb. Cortex 2014; 1–13
13. Zielinski BA, Prigge MBD, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS, Fletcher PT, Zygmunt KM, Travers BG, Lange N, Alexander AL, Bigler ED, Lainhart JE: Longitudinal changes in cortical thickness in autism and typical development. Brain 2014; 137:1799–1812

14. Foster NE V, Doyle-thomas KAR, Tryfon A, Hyde KL: Pediatric Neurology Structural Gray Matter Differences During Childhood Development in Autism Spectrum Disorder: A Multimetric Approach. 2015; 53:350–359
15. Delmonte S, Balsters JH, Mcgrath J, Fitzgerald J, Brennan S, Fagan AJ, Gallagher L: Social and monetary reward processing in autism spectrum disorders. Mol. Autism 2012; 3:7
16. Di Martino A, Kelly C, Grzadzinski R, Zuo XN, Mennes M, Mairena MA, Lord C, Castellanos FX, Milham MP: Aberrant striatal functional connectivity in children with autism. Biol. Psychiatry 2011; 69:847–856
17. Langen M, Leemans A, Johnston P, Ecker C, Daly E, Murphy CM, dell'Acqua F, Durston S, Murphy DGM: Fronto-striatal circuitry and inhibitory control in autism: Findings from diffusion tensor imaging tractography. Cortex 2012; 48:183–193
18. Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, Amaral DG: Increased Rate of Amygdala Growth in Children Aged 2 to 4 Years With Autism Spectrum Disorders. 2015; 69:53–61
19. Schumann CM, Hamstra J, Goodlin-jones BL, Lotspeich LJ, Kwon H, Buonocore MH, Lammers CR, Reiss AL, Amaral DG: (CS) The Amygdala Is Enlarged in Children But Not Adolescents with Autism; the Hippocampus Is Enlarged at All Ages-Schumann et al. 2004; 24:6392–6401
20. Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SCR: The amygdala theory of autism. Neurosci. Biobehav. Rev. 2000; 24:355–364
21. Chevallier C, Kohls G, Troiani V, Brodkin, ES, Schultz RT: The social motivation theory of autism. Trends Cogn. Neurosci. 2013; 16:231–239
22. Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM: Towards a neuroanatomy of autism: A systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur. Psychiatry 2008; 23:289–299
23. Nickl-Jockschat T, Habel U, Maria Michel T, Manning J, Laird AR, Fox PT, Schneider F, Eickhoff SB: Brain structure anomalies in autism spectrum disorder-a meta-analysis of VBM studies using anatomic likelihood estimation. Hum. Brain Mapp. 2012; 33:1470–1489
24. Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, Chisum HJ, Moses P, Pierce K, Lord C, Lincoln AJ, Pizzo S, Schreibman L, Haas RH, Akshoomoff NA, Courchesne RY: Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. Neurology 2001; 57:245–254
25. Courchesne E, Campbell K, Solso S: Brain growth across the life span in autism: Age-specific changes in anatomical pathology. Brain Res. 2011; 1380:138–145
26. Ecker C, Bookheimer SY, Murphy DGM: Neuroimaging in autism spectrum disorder: Brain structure and function across the lifespan. Lancet Neurol. 2015; 14:1121–1134
27. Ecker C, Marquand A, Mourao-Miranda J, Johnston P, Daly EM, Brammer MJ, Maltezos S, Murphy CM, Robertson D, Williams SC, others: Describing the Brain in Autism in Five Dimensions–Magnetic Resonance Imaging-Assisted Diagnosis of Autism Spectrum Disorder Using a Multiparameter Classification Approach. J. Neurosci. 2010; 30:10612
28. Uddin LQ, Menon V, Young CB, Ryali S, Chen T, Khouzam A, Minshew NJ, Hardan AY: Multivariate searchlight classification of structural magnetic resonance imaging in children and adolescents with autism. Biol. Psychiatry 2011; 70:833–841
29. Wolfers T, Buitelaar JK, Beckmann C, Franke B, Marquand AF: From estimating

activation locality to predicting disorder: a review of pattern recognition for neuroimaging-based psychiatric diagnostics. Neurosci. Biobehav. Rev. 2015;
30. Di Martino A, Connor DO, Chen B, Alaerts K, Anderson JS: Enhancing studies of the connectome in autism using the autism brain imaging data exchange II. Sci. Data 2017; 4:1–15
31. Button KS, Ioannidis JPA, Mokrysz C, Nosek BA, Flint J, Robinson ESJ, Munafò MR: Power failure: why small sample size undermines the reliability of neuroscience. Nat. Rev. Neurosci. 2013; 14:365–376
32. Khundrakpam B, Lewis JD, Evans AC, Lewis JD, Kostopoulos P, Carbonell F, Evans AC: Cortical Thickness Abnormalities in Autism Spectrum Disorders Through Late Childhood, Adolescence , and Adulthood : A Large-Scale MRI Study. Cereb. Cortex 2017; 27:1721–1731
33. Lord C, Risi S, Lambrecht L, Cook EHJ, Leventhal BL, DiLavore PC, Pickles A, Rutter M: The Autism Diagnostic Schedule – Generic: A standard measures of social and communication deficits associated with the spectrum of autism. J. Autism Dev. Disord. 2000; 30:205–223
34. Higgins JPT, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. BMJ Br. Med. J. 2003; 327:557–560
35. Benner A: Multivariable Fractional Polynomials. 2007; 1–9
36. van Erp TGM, Hibar DP, Rasmussen JM, Glahn DC, Pearlson GD, Andreassen O a, Agartz I, Westlye LT, Haukvik UK, Dale a M, Melle I, Hartberg CB, Gruber O, Kraemer B, Zilles D, Donohoe G, Kelly S, McDonald C, Morris DW, Cannon DM, Corvin A, Machielsen MWJ, Koenders L, de Haan L, Veltman DJ, Satterthwaite TD, Wolf DH, Gur RC, Gur RE, Potkin SG, Mathalon DH, Mueller B a, Preda A, Macciardi F, Ehrlich S, Walton E, Hass J, Calhoun VD, Bockholt HJ, Sponheim SR, Shoemaker JM, van Haren NEM, Pol HEH, Ophoff R a, Kahn RS, Roiz-Santiañez R, Crespo-Facorro B, Wang L, Alpert KI, Jönsson EG, Dimitrova R, Bois C, Whalley HC, McIntosh a M, Lawrie SM, Hashimoto R, Thompson PM, Turner J a: Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. Mol. Psychiatry 2015; 1–7
37. Hibar DP, Westlye LT, van Erp TGM, Rasmussen J, Leonardo CD, Faskowitz J, Haukvik UK, Hartberg CB, Doan NT, Agartz I, Dale AM, Gruber O, Krämer B, Trost S, Liberg B, Abé C, Ekman CJ, Ingvar M, Landén M, Fears SC, Freimer NB, Bearden CE, Sprooten E, Glahn DC, Pearlson GD, Emsell L, Kenney J, Scanlon C, McDonald C, Cannon DM, Almeida J, Versace A, Caseras X, Lawrence NS, Phillips ML, Dima D, Delvecchio G, Frangou S, Satterthwaite TD, Wolf D, Houenou J, Henry C, Malt UF, Bøen E, Elvsåshagen T, Young AH, Lloyd AJ, Goodwin GM, Mackay CE, Bourne C, Bilderbeck A, Abramovic L, Boks MP, van Haren NEM, Ophoff RA, Kahn RS, Bauer M, Pfennig A, Alda M, Hajek T, Mwangi B, Soares JC, Nickson T, Dimitrova R, Sussmann JE, Hagenaars S, Whalley HC, McIntosh AM, Thompson PM, Andreassen OA: Subcortical volumetric abnormalities in bipolar disorder. Mol. Psychiatry 2016; 1–7
38. Hoogman M, ENIGMA-consortium, Rubia K, Franke B: Subcortical brain volume differences between participants with ADHD and healthy individuals across the lifespan: an ENIGMA collaboration. Lancet Psychiatry 2017; 4:310–319
39. Carlisi CO, Norman LJ, Lukito SS, Radua J, Mataix-cols D, Rubia K: Comparative Multimodal Meta-analysis of Structural and Functional Brain Abnormalities in Autism Spectrum Disorder and Obsessive-Compulsive Disorder. Biol. Psychiatry 2016; epub

ahead of print
40. Shafritz KM, Bregman JD, Ikuta T, Szeszko PR: Neural systems mediating decision-making and response inhibition for social and nonsocial stimuli in autism. Prog. Neuro-Psychopharmacology Biol. Psychiatry 2015; 60:112–120
41. McAlonan G, Daly E, Kumari V, Critchley H, Van Amelsvoort T, Suckling J, Simmons A, Sigmundsson T, Greenwood K, Russell A, Schmitz N, Happé F, Howlin P, Murphy D: Brain anatomy and sensorimotor gating in Asperger's syndrome. Brain 2002; 125:1594–606
42. Weisberg J, Milleville SC, Kenworthy L, Wallace GL, Gotts SJ, Beauchamp MS, Martin A: Social Perception in Autism Spectrum Disorders: Impaired Category Selectivity for Dynamic but not Static Images in Ventral Temporal Cortex. 2014; 37–48
43. Lombardo M V, Pierce K, Campbell K, Courchesne E, Lombardo M V, Pierce K, Eyler LT, Barnes CC, Ahrens-barbeau C: Different Functional Neural Substrates for Good and Poor Language Outcome in Autism Article Different Functional Neural Substrates for Good and Poor Language Outcome in Autism. Neuron 2015; 86:567–577
44. Wierenga L, Langen M, Ambrosino S, van Dijk S, Oranje B, Durston S: Typical development of basal ganglia, hippocampus, amygdala and cerebellum from age 7 to 24. Neuroimage 2014; 96:67–72
45. Langen M, Schnack HG, Nederveen H, Bos D, Lahuis BE, de Jonge M V., van Engeland H, Durston S: Changes in the Developmental Trajectories of Striatum in Autism. Biol. Psychiatry 2009; 66:327–333
46. Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, Vauss YC, Rapoport JL: Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: Ages 4-18 years. J. Comp. Neurol. 1996; 366:223–230
47. Østby Y, Tamnes CK, Fjell AM, Westlye LT, Due-Tønnessen P, Walhovd KB: Heterogeneity in Subcortical Brain Development: A Structural Magnetic Resonance Imaging Study of Brain Maturation from 8 to 30 Years. J. Neurosci. 2009; 29:11772–11782
48. Baron-Cohen S, Knickmeyer R, Belmonte MK: Sex differences in the brain: Implications for explaining autism. Science (80-.). 2005; 310:819–823
49. Ecker C, Andrews DS, Gudbrandsen CM, Marquand AF, Ginestet CE, Daly EM, Murphy CM, Lai M, Lombardo M V, Ruigrok AN V, Bullmore ET, Suckling J, Williams SCR, Baron-cohen S, Craig MC, Murphy DGM: Association Between the Probability of Autism Spectrum Disorder and Normative Sex-Related Phenotypic Diversity in Brain Structure. 2017; 329–338

Tables:

	Controls	ASD	group difference
N	1651	1571	
Age (mean)	15.83	15.41	-
Age (SD)	8.41	8.64	-
Age (range)	2-56	2-64	-
Females (N)	393	224	Controls > ASD***
Females (%)	23.8	14.25	Controls > ASD***
IQ (Mean)	111	103	Controls > ASD***
IQ (Range)	80-149	65-123	
Medication use (N)	0	233	
Comorbidities (N)	0	148	

Table 1. Demographics of all participants. *** p-value <.001. Medication use indicates any type of current medication use, regardless of duration. Comorbidities indicate any type of current comorbid psychiatric disorders.

Subcortical	N controls	N patients	Diagnosis (Cohen's d)	Diagnosis (p-FDR)	Age (Cohen's d)	Age (p-FDR)	Age * Diagnosis (p-FDR)	Sex (Cohen's d)	Sex (p-FDR)	IQ (Cohen's -d)	IQ (p-FDR)
Lateral Ventricles	1569	1482	**0.11**	**0.01**	**0.16**	**0.001**	0.529	-0.096	0.064	**0.17**	**0.050**
Thalamus	1597	1494	0.00	0.989	**0.07**	**0.042**	0.654	**-0.111**	**0.034**	0.01	0.110
Caudate	1604	1517	-0.05	0.206	**-0.19**	**0.001**	0.230	**-0.154**	**0.003**	0.00	0.150
Putamen	1600	1518	**-0.10**	**0.013**	**-0.12**	**0.001**	0.882	**-0.218**	**<0.001**	0.00	0.140
Pallidum	1596	1509	**-0.08**	**0.046**	**-0.26**	**0.001**	0.261	0.01	0.839	0.00	0.140
Hippocampus	1595	1507	-0.05	0.222	**0.14**	**0.001**	0.521	-0.053	0.310	0.02	0.100
Amygdala	1601	1508	**-0.08**	**0.046**	**0.13**	**0.001**	0.230	**-0.163**	**0.002**	0.01	0.110
Nucleus accumbens	1596	1518	**-0.13**	**0.002**	**-0.19**	**0.001**	0.230	**-0.218**	**<0.001**	0.13	0.060
ICV	1606	1522	**0.13**	**0.009**	**0.14**	**0.001**	0.261	**-0.239**	**<0.001**	0.02	0.100
Frontal											
Superior Frontal	1574	1657	**0.17**	**0.0000**	**-0.30**	**<.0001**	**0.000**	**0.08**	**0.0386**	-0.03	0.7312
Rostral Middle Frontal	1572	1658	**0.20**	**0.0000**	**-0.39**	**<.0001**	**0.000**	0.03	0.3790	-0.02	0.8178
Caudal Middle Frontal	1574	1656	**0.08**	**0.0567**	**-0.24**	**<.0001**	**0.000**	**0.08**	**0.0253**	0.05	0.3965
Pars Triangularis	1572	1653	**0.11**	**0.0056**	**-0.31**	**<.0001**	**0.000**	0.03	0.3418	-0.01	0.8178
Pars Orbitalis	1573	1656	**0.12**	**0.0023**	**-0.28**	**<.0001**	**0.000**	0.03	0.4900	-0.07	0.3189
Pars Opercularis	1572	1654	0.01	0.7333	**-0.25**	**<.0001**	**0.002**	0.00	0.9297	0.00	0.9818
Medial Orbitofrontal	1572	1656	**0.15**	**0.0003**	**-0.37**	**<.0001**	**0.000**	-0.03	0.4060	-0.09	0.1432
Lateral Orbitofrontal	1574	1655	0.05	0.2941	**-0.32**	**<.0001**	**0.000**	-0.03	0.3670	-0.02	0.8050
Precentral	1569	1655	-0.06	0.1319	**-0.10**	**0.006**	0.544	0.04	0.2386	**0.12**	**0.0298**
Paracentral	1574	1653	-0.01	0.7333	**-0.29**	**<.0001**	**0.000**	0.02	0.4919	0.06	0.3621
Frontal Pole	1568	1655	**0.10**	**0.0109**	**-0.25**	**<.0001**	**0.000**	-0.02	0.5670	-0.02	0.8050
Insula											

Insula	1567	1651	-0.07	0.1133	**-0.13**	**0.0007**	0.454	-0.05	0.1950	-0.02	0.8178
Cingulate											
Rostral Anterior (Frontal)	1569	1652	0.02	0.5851	**-0.20**	**<.0001**	**0.008**	0.02	0.5408	-0.08	0.2284
Caudal Anterior (Frontal)	1566	1651	0.03	0.5226	**-0.16**	**<.0001**	**0.012**	0.05	0.1972	-0.08	0.2284
Posterior (Parietal)	1568	1657	**0.13**	**0.0021**	**-0.22**	**<.0001**	**0.002**	0.02	0.6343	-0.06	0.3754
Isthmus (Parietal)	1572	1650	**0.08**	**0.0436**	**-0.21**	**<.0001**	**0.001**	-0.02	0.5424	-0.02	0.7873
Parietal											
Superior Parietal	1572	1654	-0.01	0.8190	**-0.34**	**<.0001**	**0.000**	0.06	0.1071	-0.01	0.8460
Inferior Parietal	1572	1657	0.02	0.6392	**-0.32**	**<.0001**	**0.000**	0.01	0.7128	-0.02	0.8050
Supramarginal	1572	1652	-0.07	0.0785	**-0.27**	**<.0001**	**0.000**	0.00	0.9924	0.03	0.7258
Postcentral	1569	1655	-0.03	0.5226	**-0.25**	**<.0001**	**0.000**	0.03	0.4667	0.02	0.7873
Precuneus	1573	1658	0.03	0.5261	**-0.34**	**<.0001**	**0.000**	0.04	0.2871	-0.06	0.3621
Temporal											
Superior temporal	1569	1652	-0.08	0.0623	-0.04	0.3132	0.387	0.00	0.9417	0.05	0.3965
Middle temporal	1570	1653	**-0.10**	**0.0141**	**-0.14**	**0.0002**	0.150	0.02	0.5395	0.07	0.3189
Inferior temporal	1571	1655	**-0.14**	**0.0008**	**-0.15**	**<.0001**	0.044	-0.05	0.1544	-0.05	0.4532
Banks of the Superior Temporal Sulcus	1562	1647	-0.03	0.5226	**-0.15**	**<.0001**	0.098	-0.02	0.5548	0.03	0.7873
Fusiform	1570	1655	**-0.19**	**0.0000**	**-0.17**	**<.0001**	**0.024**	-0.06	0.0967	-0.01	0.9140
Transverse Temporal	1574	1655	**-0.12**	**0.0026**	**-0.16**	**<.0001**	**0.039**	**0.09**	**0.0100**	0.05	0.3965
Entorhinal	1559	1641	**-0.21**	**0.0000**	0.00	0.9465	0.683	-0.01	0.8227	0.05	0.4532
Temporal Pole	1565	1648	**-0.13**	**0.0013**	0.03	0.4236	0.481	-0.03	0.3445	0.03	0.7312
Parahippocampal	1569	1653	**-0.10**	**0.0144**	-0.06	0.1230	0.556	0.05	0.1567	0.05	0.4532
Occipital											
Lateral Occipital	1569	1652	-0.05	0.2023	**-0.21**	**<.0001**	**0.000**	-0.02	0.5264	-0.02	0.7873
Lingual	1572	1655	-0.04	0.4167	**-0.24**	**<.0001**	**0.000**	-0.03	0.4777	-0.01	0.8826
Cuneus	1571	1651	0.07	0.0858	**-0.27**	**<.0001**	**0.000**	0.01	0.7860	-0.07	0.2784
Pericalcarine	1571	1648	0.00	0.9335	**-0.11**	**0.0029**	0.150	0.05	0.1596	0.00	0.9869

Table 2. Mega-analysis ASD v Control comparison model outcomes, including polynomial effects of age and IQ, as well as fixed effects for sex and a random effect for scan-site in the main regression model. All subcortical volumes are corrected for total ICV. All cortical thickness values are corrected for mean cortical thickness. Bold values indicate significant effects (p-values are FDR corrected). No significant effects on cortical surface area were observed. For the effect of Age, positive d-values indicate increasing volumes with higher age. For the effect of Sex, negative d-values indicate larger values in males. For the effect of IQ, positive d-values indicate a higher volume associated with higher IQ.

Figures:

Figure 1. Distributions of Age and IQ within the full sample. Blue bars represent frequencies within healthy controls, red bars in subjects with ASD. Purple bars indicate the overlap between the distributions.

Figure 2. Mega-analysis of ASD vs Control comparison, visualizing effect sizes for all subcortical and cortical partitions. (A/B) show the medial and lateral view of the striatum. (C/D) show the medial and lateral view of cortical thickness. Yellow/red hues indicate higher d-values, corresponding to larger volumes in patients with ASD. Blue hues indicate lower volumes in subjects with ASD. Images are in MNI-152 space.

Figure 3. Fractional polynomial best model fits for age for frontal cortical thickness (A), temporal cortical thickness (B), subcortical volumes (C) with significant diagnosis and age and/or age*diagnosis effects, as well as total ICV and total cortical thickness (D). . Separate fits shown for both subjects with ASD and healthy Controls.